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Viola serrata (Violaceae), a new species from Guangxi, China*

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Abstract: A new species of *Viola* (Violaceae), *V. serrata* Yan S. Huang & Q. Fan, is described from Guangxi Zhuang Autonomous Region, China. Based on field surveys, detailed morphological comparisons, and phylogenetic analyses of ITS sequences, the new species is placed within *Viola* section *Plagiostigma* subsection *Diffusae*. Morphologically, *V. serrata* is most similar to *V. changii* J.S. Zhou & F.W.Xing, but can be clearly distinguished by several key diagnostic features: the leaf margins are serrate with acute apices (vs. crenate with obtuse apices), the rhizomes are robust and well-developed (vs. a primarily fibrous root system), the petal bases feature yellow spots(vs. white), and both the ovary and the abaxial surfaces of the anterior petals are pubescent (vs. glabrous). This discovery clarifies the species boundaries within subsection *Diffusae* and provides new insights into the diversity and evolutionary history of the genus *Viola* in the karst regions of South China.

Key words: *Viola*; new species; Guangxi Zhuang Autonomous Region; phylogeny; ITS

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The genus *Viola* L. (Violaceae) is one of the most species-rich genera of angiosperms, comprising approximately 650 species distributed across diverse ecosystems worldwide (Huang et al., 2025). According to the comprehensive global phylogenetic and taxonomic revision by Marcussen et al. (2022), the genus is organized into an infrageneric framework that reflects complex evolutionary trajectories, including widespread polyploidy and long-distance dispersal events. China is recognized as a major center of diversity for the genus (Ballard et al., 1998), particularly for section *Plagiostigma* Godr., which exhibits a remarkable degree of endemism in the East Asian monsoon region (Nakamura et al., 2014; Marcussen et al., 2022).

Within sect. *Plagiostigma* Godr., subsection *Diffusae* (W. Becker) Chang represents a taxonomically well-defined lineage. As circumscribed by Marcussen et al. (2022), members of this subsection are diagnostic by their long aboveground stolon bearing 1−2 leaves and an apical rosette, stipules that are adnate to the petiole in the lower third, and a style that is

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margined and bilobate at the apex. Their corollas are typically pale pink to pale violet, often featuring a distinct greenish throat (Marcussen et al., 2022; Huang et al., 2025).

The karst landscapes of South China, characterized by high habitat heterogeneity, serve as a center for the diversification of subsect. *Diffusae*. The complex topography of this area functions as a driver for lineage diversification, fostering the emergence of specialized taxa in limestone micro-habitats. This is evidenced by the recent descriptions of *V. xinchengensis* (Wei et al., 2025) and *V. pendulipes* (Huang et al., 2023). These discoveries expand the known morphological boundaries of the subsection and highlight the profound role of the South China karst in the evolutionary radiation of *Viola*.

During field surveys in the karst hills of Yizhou, Guangxi, an unknown *Viola* species was collected. Morphologically, this plant resembles *V. changii* but differs in several constant characters: the leaf margins are serrate with acute apices (vs. crenate with obtuse apices), the rhizomes are robust and well-developed (vs. a primarily fibrous root system), the petal bases feature yellow spots (vs. white), and both the ovary and the abaxial surfaces of the anterior petals are pubescent (vs. glabrous). Phylogenetic analysis of internal transcribed spacer (ITS) sequences places this population within subsection *Diffusae* as a distinct lineage. In this study, we describe this taxon as a new species, *V. serrata*, provide a comparison with closely related species, and assess its conservation status within the karst ecosystem.

1 Materials and methods

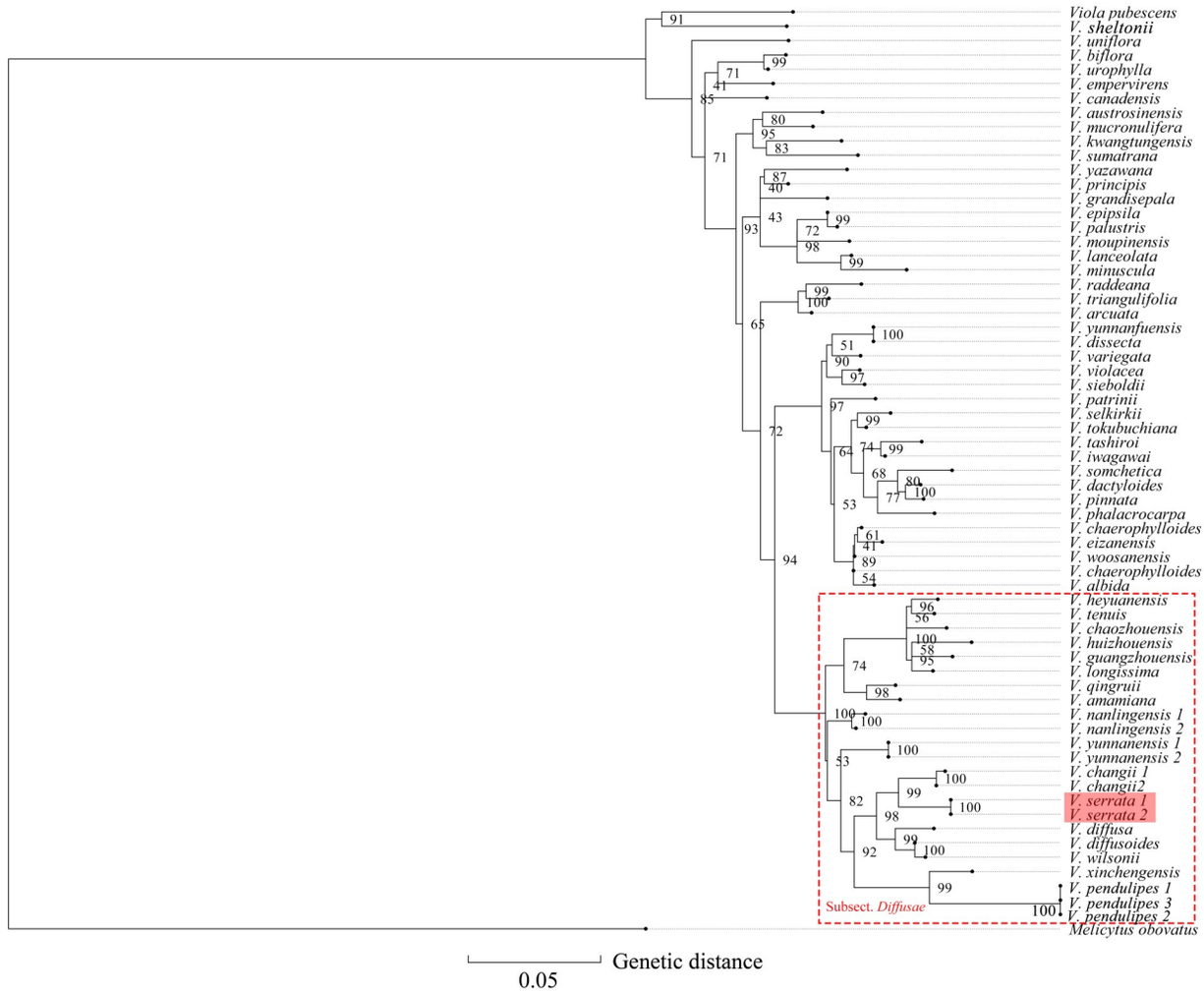
Field investigations were conducted during the flowering and fruiting periods of the putative new species in Yizhou, Guangxi, China. Representative specimens were collected and silica-gel dried for molecular analysis, with morphological data supplemented by examination of closely related taxa. All available specimens of subsect. *Diffusae* deposited in major Chinese herbaria (IBSC, IBK, GXMG, SYS) were examined, with type specimens and historically identified collections critically reviewed and photographed. High-resolution digital images were obtained from JSTOR Global Plants and GBIF. Macromorphological traits (e. g., leaf length/width, petiole length) were measured directly using a scale ruler with a color chart (precision: 1 mm). Micromorphological features (e. g., leaf serration structures) were observed and captured using a Sony α6000 camera equipped with a 100 mm macro lens (1∶2.8 reproduction ratio), with a millimeter scale bar placed adjacent to each specimen during photography.

Total genomic DNA was extracted using the modified CTAB method (Doyle et al., 1987), and the ITS region was amplified using universal primers ITS1 and ITS4 (White et al., 1990) following the protocol of Huang et al. (2021). To determine the phylogenetic position of the new species, ITS sequences of related taxa were downloaded from GenBank (Supply: Appendix table 1). Taxon sampling was designed to comprehensively represent subsect. *Diffusae*, including all currently recognized species, together with the morphologically similar species *V. changii*. Representative species from other subsections of sect. *Plagiostigma* were also included to represent the phylogenetic diversity of the section. Sequences were aligned using MAFFT v. 7.402 (Katohet et al., 2013) and Clustal W 1.8 (Thompson et al., 1994), with consistency cross-verified between methods. The best-fit nucleotide substitution model, SYM+I+R2, was selected by Model Finder (Kalyaanamoorthy et al., 2017) based on the Bayesian Information Criterion (BIC). Phylogenetic reconstruction was performed via Maximum Likelihood (ML) using IQ-TREE 2.0.3 (Minh et al., 2019) with 2 000 bootstrap replicates to evaluate topological support, and *Melicytus obovatus* was designated as the outgroup.

2 Results

2.1 Phylogeny

The aligned length of our ITS sequences was 940 bp in total. The putative new species belongs to the monophyletic *Viola* subsect. *Diffusae* (BS=100%, Fig. 1), and forms a sister group with *V. changii* (BS=99%).



With bootstrap values (BS) shown at the nodes.

Fig. 1 Phylogenetic tree of the new species and related species based on ML methods

2.2 Taxonomic treatment

Viola serrata Yan S. Huang & Q. Fan, sp. nov. (Fig.2−4)

1）Diagnosis. This species is morphologically similar to *V. changii* (Table 1), but can be distinguished by its serrate leaf margins with acute apices (vs. crenate margins with obtuse apices), anterior petals externally pubescent, with the spur of the anterior petal minutely puberulent both internally and externally (vs. glabrous), petals with yellow spots at the base (vs. white), a pubescent ovary (vs. glabrous), and a robust, well-developed rhizome (vs. a primarily fibrous root system).

2）Type. China. Guangxi Zhuang Autonomous Region: Hechi City, Yizhou District, Longtou Township, Baishantong Village, limestone cliff, 250 m elev., 24.50°N, 108.12°E, 13 Apr 2026, Yan Shuang Huang HYS 26041301 (holotype: SYS! 00237146, Fig. 2; isotype: SYS! 00237144).

3） Additional specimens examined. China. Guangxi Zhuang Autonomous Region: Hechi City, Yizhou District, Longtou Township, limestone area, 366 m elev., 24.50°N, 108.13° E, 13 May 2020, Zu-zai Lan, Ping Yang & Ji-jun Yao. 451281200513007LY (GXMG 0222986, GXMG 0222987; Guangxi Botanical Garden of Medicinal Plants).

4）Etymology. The specific epithet refers to the serrate leaf margins of the species.

5）Vernacular name. We propose the Chinese name Jù Chǐ Jǐn Cài (锯齿堇菜), referring to its serrate leaf margins.

6）Description. Perennial, stoloniferous herb, 4−7 cm tall. Rhizome obliquely ascending, pale white, 1−2.5 cm long, densely covered with remains of



Fig. 2 Holotype of *Viola serrata* sp. nov. (SYS00237146)

stipules and petioles. Stems lateral, pubescent, creeping or ascending, 2−5 cm long, purplish-brown, producing an apical rosette of leaves at the tip. Stipules ca. 4 mm long, adnate to the petiole for about 2 mm at the base, linear-lanceolate, apex acute, margins with sparse long fimbriae, pubescent. Petioles 0.5−3.0 cm long, nearly wingless, densely pubescent. Leaf blades oblong, (1.1−2.8) cm× (0.6−1.6) cm, densely pubescent on both surfaces; margin serrate; base truncate; apex acuminate.

Chasmogamous flowers ca. 1.2 cm in diameter; peduncles slender, 1.5−3.0 cm long, pubescent, with two opposite bracteoles above the middle; bracteoles linear to lanceolate, ca. 6 mm long, apex obtuse. Sepals green, pubescent, with purple spots becoming sparser from base to apex, lanceolate; unequal in length, adaxial (upper) three sepals slightly longer, ca. 4.2 mm×1.5 mm, abaxial (lower) two sepals ca. 3.8 mm×1.5 mm; margins entire and hyaline, apex acuminate, base truncate, with extremely short appendages.

Petals purple; anterior petal with conspicuous violet veins; posterior and lateral petals with a yellow to green patch at the base and glandular at base. Poste-

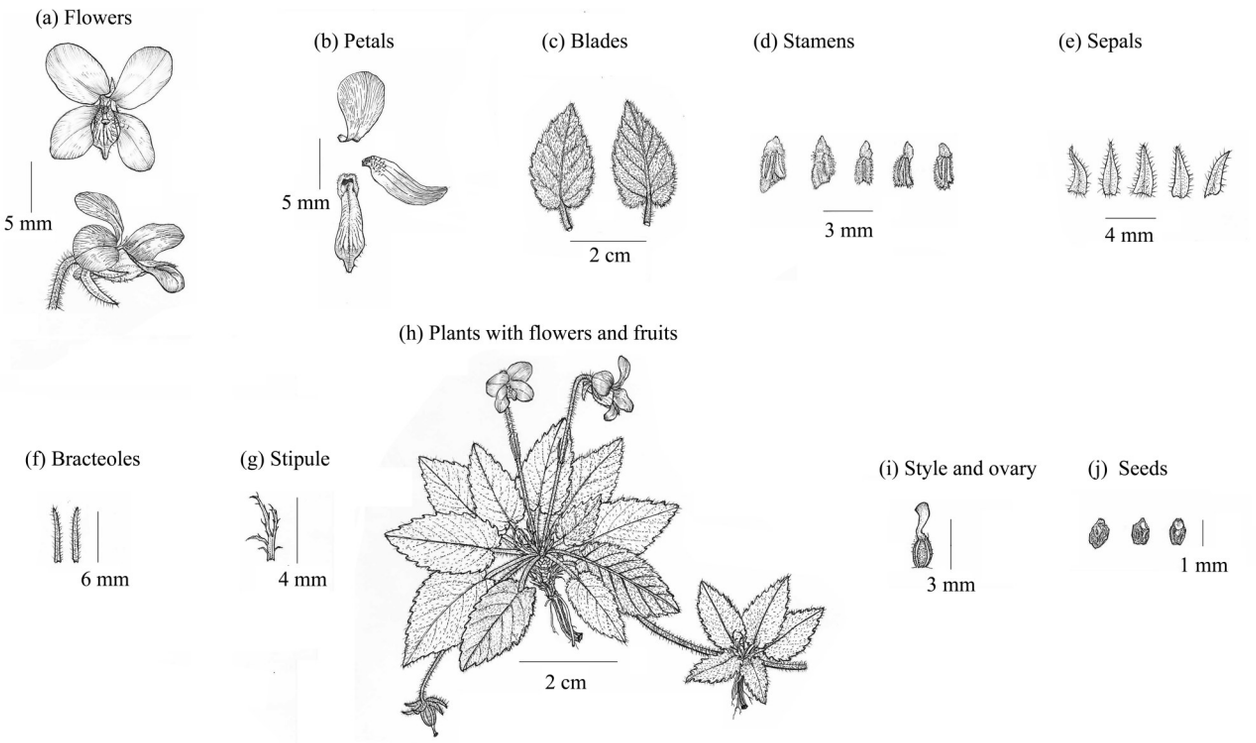


Fig. 3 *Viola serrata* sp. nov

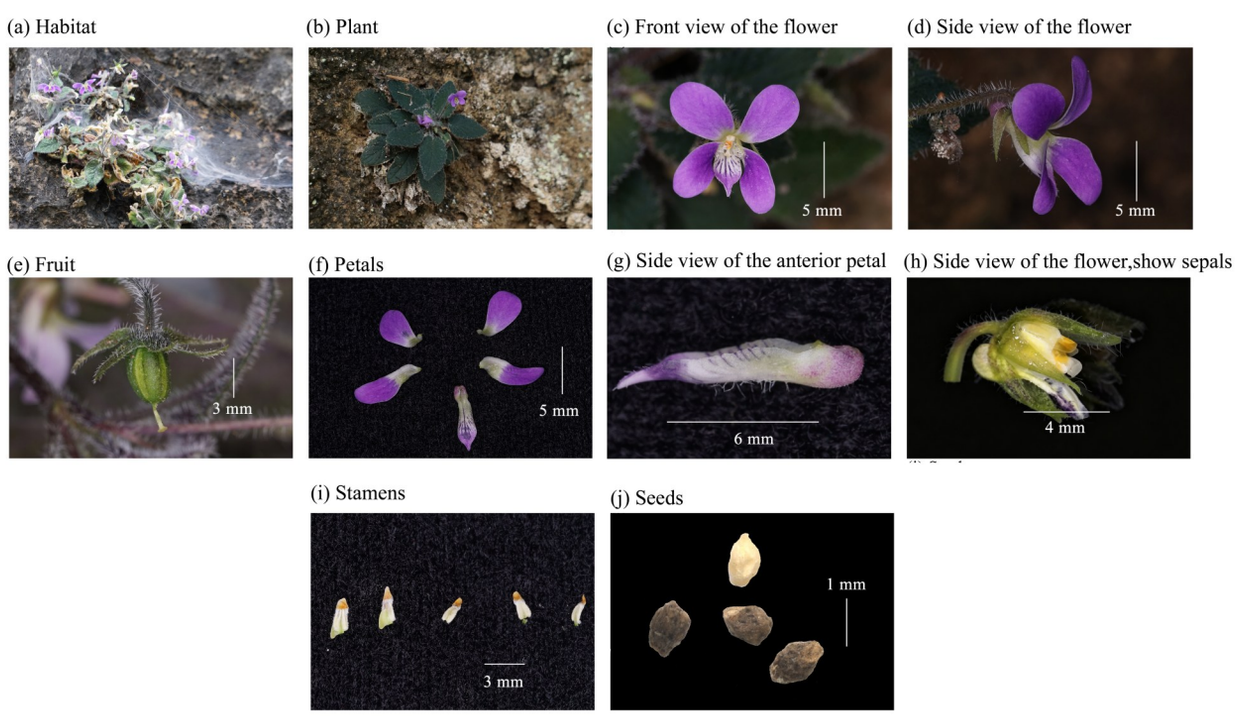


Fig. 4 *Viola serrata* sp. nov.

rior petals narrowly ovate, ca. 7 mm long, margin entire, apex obtuse; lateral petals ca. 9 mm long, margin entire, apex obtuse to erose; anterior petal narrowly oblong, externally pubescent, with a short saccate spur at base, spur puberulent both inside and outside, including spur ca. 9 mm long, margin entire, apex acuminate.

Stamens 5, unequal, puberulent; anther thecae 2.5−3.0 mm long, terminal appendages ca. 1 mm long; nectar spurs of the two anterior stamens ca. 1.6

Table 1 Morphological differences between *Viola serrata* and *V. changii*

Species	*V. serrata*	*V. changii*
Leaves	Oblong，(1. 1−2. 8) cm×(0. 6−1. 6) cm，margin serrate，apex acuminate，base truncate，both surfaces densely pubescent	Ovate or ovate-orbicular，1. 5 cm×1. 2 cm，margin obtusely crenate or subentire，apex obtuse，base narrowly cordate，abaxially puberulous only along veins，adaxially densely puberulous
Pedicel	1. 5−3 cm long，pubescent	6−8 cm long，puberulous，
Flower	Purple，ca. 1. 2 cm in diameter；anterior petal externally pubescent；spur puberulent both inside and outside	White to pale purple，1. 8−2. 2 cm across；anterior petal glabrous externally；spur glabrous
Petal base spot	Yellow to green	White
Ovary	Sparsely pubescent	Glabrous
Root system	Robust，well-developed rhizome（obliquely ascending）	Primarily fibrous root system

mm long, triangular. Ovary ovoid to ellipsoid, ca. 1.5 mm long, sparsely pubescent; style clavate, ca. 1.8 mm long, conspicuously geniculate at the base; stigma glabrous, with thickened lateral margins, apex shortly beaked. Capsules ellipsoid, 3−5 mm long, light green, puberulent, longitudinally grooved, 3-valved when mature. Seeds brown, oblongoid, ca. 1 mm long; elaiosomes absent.

7）Distribution and habitat. Currently known only from Yizhou District, Hechi City, Guangxi Zhuang Autonomous Region, China. The species grows on limestone cliffs at elevations of 250−366 m.

8）Conservation status. Based on current field observations, fewer than 50 mature individualswere recorded from a single roadside cliff. According to the IUCN Red List Categories and Criteria (IUCN 2025), the species is assessed as Critically Endangered (CR B_2ab (iii)).

3 Discussion

Like its closely related species *Viola changii* and other lithophytic taxa such as *V. guangzhouensis* A.Q. Dong, J.S.Zhou & F.W.Xing and *V. huizhouensis* Yan S. Huang & Q. Fan (Dong et al., 2009; Huang et al., 2021), *V. serrata* is densely pubescent throughout the plant. In these limestone-dwelling species, dense pubescence may represent an adaptation to reduce water loss under exposed and drought-prone conditions of karst habitats (Watson et al., 2019). However, *V. serrata* further differs by having pubescence on the external surface of the anterior petal and spur, whereas in related species the petals are usually glabrous externally or hairy only on the inner surface. During field investigations, numerous red mites (Tetranychidae) were observed on the flowers, and many flowers remained open despite being covered by fine webs (Fig. 4a). We speculate that the unusual external pubescence of the petals may function as a mechanical barrier against small arthropods, thereby reducing floral damage and maintaining reproductive function. This interpretation is consistent with previous studies demonstrating that plant trichomes can significantly reduce mite colonization and feeding success (Walter, 1996).

Phylogenetically, ITS sequences successfully resolved *V. serrata* as a distinct lineage sister to *V. changii*, with strong bootstrap support (Fig. 1). This result is consistent with the clear morphological differentiation between the two taxa and supports the recognition of the new species within subsection *Diffusae*. Although ITS markers are known to have limited resolution for deeper infrageneric relationships in *Viola*, largely due to hybridization and incomplete lineage sorting (Marcussen et al., 2022), they remain effective for species-level delimitation in recently diverged lineages. In the present study, ITS provided sufficient discriminatory power to separate closely related taxa within subsection *Diffusae* (Huang et al., 2023; Wei et al., 2025), especially when integrated with detailed morphological evidence.

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广西堇菜属一新种:锯齿堇菜

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摘要:报道了产自广西壮族自治区的堇菜科(Violaceae)堇菜属(*Viola*)一个新种——锯齿堇菜(*Viola serrata* Yan S. Huang & Q.Fan)。通过形态学比较及nrDNA ITS区序列的系统发育分析,确认该种隶属于堇菜属*Plagiostigma*组*Diffusae*亚组。锯齿堇菜与张氏堇菜(*V. changii* J.S. Zhou & F.W. Xing)亲缘关系较近,但可通过叶缘具锯齿且先端锐尖、全株密被柔毛、具粗壮发达的根茎,花瓣基部具黄色斑点和下瓣背面被毛等特征与后者明显区分;系统发育分析结果也支持其作为独立物种的分类地位。本研究为*Diffusae*亚组的分类修订和系统演化研究提供了新的证据,并进一步丰富了中国南方地区植物区系的多样性。

关键词:堇菜属;新种;广西壮族自治区;系统发育;ITS序列

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